AEROMONAS HYDROPHILA and *AEROMONAS SOBRIA* AS POTENTIAL FOOD POISONING SPECIES: A REVIEW[1]

ABSTRACT

Epidemiological and toxicological evidence implicating Aeromonas hydrophila *and* Aermononas sobria *as agents of human gastroenteritis is reviewed. These psychrotrophic species are common contaminants of refrigerated animal products, and the possibility that they may cause food poisoning is discussed.*

INTRODUCTION

It is generally recognized that the majority of confirmed food poisoning cases occurring in the United States are attributable to either *Staphylococcus aureus, Clostridium perfringens,* or *Salmonella.* While this statistic is accurate, it tends to be misleading in that 56–70% of the known or suspected food poisoning outbreaks are classified as being of unknown etiology (Center for Disease Control 1977). Some of these cases undoubtedly reflect incomplete investigations that have not allowed confirmation of one of the three major food poisoning bacteria. However, it has been generally assumed that substantial portion of these unidentified food poisoning cases are caused by species not routinely assessed during investigations of suspected outbreaks. During the past 10 years, there has been renewed interest in identifying the causes of human gastroenteritis, particularly on the part of clinical microbiologists. This has resulted in increased understanding of the causes of diarrheal diseases, particularly in regard to the roles enterotoxins and other virulence factors play in inducing a disease response. This has led to a significant expansion of the list of bacteria classified as potential food

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poisoning species, which now includes such organisms as *Campylobacter jejuni,* enteropathogenic *Escherichia coli, Yersinia enterocolitica, Vibrio parahemolyticus, Bacillus cereus,* and *Listeria monocytogenes.* The purpose of the present manuscript is to review recent findings pertaining to two additional species, *Aeromonas hydrophila* and *Aeromonas sobria,* that appear to be pathogens of food safety significance.

Taxonomy

The majority of the studies assessing the significance of *Aeromonas* species as agents of human gastroenteritis have concentrated on the role of *A. hydrophila.* However, in most instances this actually represented the *A. hydrophila* group, or more simply the motile aeromonads. This general lack of detailed differentiation of species designations in large part, reflects the current state of flux in regard to the taxonomy of the genus. In the 8th edition of Bergey's Manual (Schubert 1974), the genus *Aeromonas* was subdivided into three species, *A. hydrophila, A. punctata,* and *A. salmonicida. A. salmonicida* was readily differentiated on the basis of being nonmotile, incapable of growth at 37°C, indole-negative, and capable of producing a brown diffusable pigment on trypticase soy agar. The differentiation of *A. hydrophila* and *A. punctata* was less clearcut, and these species were subdivided into three and two subspecies, respectively. Further, *A. hydrophila* subsp. *hydrophila* and *A. hydrophila* subsp.*anaerogenes* were additionally subdivided into biotypes based on Voges-Proskauer reaction and the ability to ferment gluconic acid.

Popoff and Veron (1976) re-examined the genus and recommended its reorganization into a more straightforward taxonomy. These changes have been largely adopted by reseachers, and are reflected in the most recent edition of Bergey's Manual (Popoff 1984). Currently, the genus is defined to include bacteria that are gram-negative, rod-shaped (straight), nonmotile or motile by a single polar flagellum, facultatively anaerobic, capable of reducing nitrate to nitrite, oxidase-positive, catalase-positive, and vibriostatic agent 0/129 (2,4-diamino-6,7-diisopropylpteridine) resistant, with a G + C mol % of 57–63. Within the genus, four species are recognized; *A. hydrophila, A. salmonicida, A. sobria,* and *A. caviae. A. salmonicida* is additionally subdivided into three subspecies; *salmonicida, achromogenes,* and *masoucida.*

A. salmonicida is differentiated from other *Aeromonas* species by lack of motility and inability to grow in nutrient broth at 37°C. Differentiation among the motile species is more complex, relying on the typical responses for a number of biochemical characteristics (Table 1). In addition, the motile aeromonads are considered universally positive for starch hydrolysis, leci-

Table 1. Characteristics used to differentiate the motile aeromonads (from Popoff).

CHARACTERISTIC	DIFFERENTIATION OF MOTILE AEROMONAS SPECIES		
	A. hydrophila	A. caviae	A. sobria
Esculin hydrolysis	+	+	-
Growth in KCN broth	+	+	-
Histidine utilization	+	+	-
Arginine utilization	+	+	-
Arabinose utilization	+	+	-
Salicin fermentation	+	+	-
Voges-Proskauer	+	-	d
Gas from glucose	+	-	+
H_2S from cysteine	+	-	+

thinase, phosphatase, ADH, ONPG (o-nitrophenyl-B-D-galactopyranoside) hydrolysis, growth in nutrient broth without NaCl, and fermentation of trehalose, fructose, galactose, and dextrin. The current edition of Bergey's (Popoff 1984) further lists the motile aeromonads as being universally negative for pectinase, ODC, tryptophan, and phenylalanine deaminases, growth on cetrimide agar, growth in nutrient broth with 5% NaCl, and acid production from sorbose, erythritol, and raffinose. These characteristics can be useful in differentiating the motile *Aeromonas* from species of other genera.

Epidemiology

Members of the genus *Aeromonas* are well-known as pathogens of fish (Shotts *et al.* 1972; Esch *et al.* 1976; Miller and Chapman 1976), amphibia (Rigney *et al.* 1978), and reptiles (Gordon *et al* 1979; Shotts *et al.* 1972), and are additionally recognized as important causes of septicemia in immunocompromised humans. However, it was not until the 1970's that a substantial number of reports began to appear in the literature implicating members of the *A. hydrophila* group, particularly *A. hydrophila* and *A. sobria*, as possible worldwide agents of gastroenteritis in humans (Echeverria

et al. 1981; Champsaur *et al.* 1982; Kalina 1977; Wadström *et al.* 1976; Rahman and Willowghby 1980; Rosner 1964; Sanyal *et al.* 1975; Pitarangsi *et al.* 1982; Gelbart and Prabhudesai 1984). This has included the species having been implicated as agents of travelers' and infantile diarrhea, as well as being suspected causes of foodborne and waterborne gastroenteritis outbreaks.

Surveys of fecal samples from individuals suffering from gastroenteritis symptoms support the contention that members of genus *Aeromonas* are significant enteric pathogens. These analyses have indicated that *A. hydrophila* is isolated at approximately the same rate as other important enteric pathogens such as *Salmonella* and *Campylobacter jejuni* (von Gravenitz and Zinterhofer 1970; Trust and Chipman 1979; Goodwin *et al.* 1983; Echeverria *et al.* 1983; Millership *et al.* 1983; Himmelreich *et al* 1984; Beyt *et al* 1984; Bechtel and Campos 1984). For example, Goodwin *et al.* (1983) reported that the fecal carriage rate of enterotoxigenic *A. hydrophila* among 500 pediatric patients displaying diarrheal symptoms was approximately 10%, while the rate in a matched control group free of symptoms was only 0.4%. A similar pattern was observed with adults, though the results were less clearcut in that the fecal carriage rate among asymptomatic individuals was higher. Studying the inhabitants of a village in Thailand over the course of a year, Echeverria *et al.* (1983) observed a strong temporal correlation between the incidence of gastroenteritis and the isolation of fecal *A. hydrophila*. They suggested that the housefly was a probable vector for transmission of the microorganism. Overall, the results from available case studies and surveys suggest strongly that at least some strains of *A. hydrophila* and *A. sobria* can cause gastroenteritis episodes ranging in severity from mild diarrheal outbreaks to life-threatening cholera-like manifestations. Little specific information is available concerning the role that food may play in relation to the incidence of *Aeromonas* gastroenteritis.

Toxicology

In addition to epidemiological indications that members of the genus *Aeromonas* can be involved in human gastroenteritis outbreaks, laboratory studies evaluating the enterotoxigenicity of the microorganism are also indicative of an enteric pathogen. Using various assays for enterotoxigenicity such as ligated ileal loop (rabbit), suckling mouse, vascular permeability, or cell culture toxicity, a number of investigators have concluded that *A. hydrophila* and *A. sobria* strains may produce one or more enterotoxins. Pitarangsi *et al.* (1982) reported that approximately 70% of clinical isolates examined were positive for the production of a cytotoxic factor against cell cultures. Boulanger *et al.* (1977) reported similar findings for *A. hydrophila* and *A. sobria* isolated from healthy and diseased fishes. Jiwa (1983) reported equivalent percentages of enterotoxin-positive isolates among clini-

cal, environmental, and fish sources. Turnbull *et al.* (1984) examined isolates from clinical and environmental sources, and found that 95% and 94% of the *A. hydrophila* and *A.sobria*, respectively, were enterotoxin-positive, while only 11% of the isolated *A. cavaie* were enterotoxin-positive. Turnbull *et al.* (1984) suggested that there was no apparent correlation between enterotoxigenicity and clinical symptoms, since 41% of the *Aeromonas* isolated from diarrheal stools were enterotoxin-negative. Conversely, Burke *et al.* (1984a) reported a strong association (91%) between enterotoxigenicity and gastroenteritis isolates. Additional research is needed to assess and clarify the significance of enterotoxin synthesis in relation to the virulence of *Aeromonas* isolates.

At the present time, there is a substantial amount of confusion concerning the identity of the enterotoxin(s) produced by *Aeromonas* species. Most isolates are cytotoxic to cell cultures, producing responses similar to those noted with *Clostridium perfringens* enterotoxin (Hostacka *et al.* 1982; Cumberbatch *et al.* 1979; Jiwa 1983). Additionally, a number of investigators have reported that some isolates of *Aeromonas* elaborate a heat-labile, cytotonic enterotoxin that has activity similar to that noted with cholera or *E. coli* heat-labile enterotoxins (Ljungh *et al.* 1981, 1982a, 1982b; Ljungh and Wadström 1983; Jiwa 1983; Turnbull *et al.* 1984; Campbell *et al.* 1984). Ljungh and coworkers (Ljungh *et al.* 1981, 1982a, 1982b; Ljungh and Wadström 1983) isolated two hemolysins and a distinct enterotoxin from *A. hydrophila*. They found that the enterotoxin elicited responses similar to those noted with cholera toxin; however, the activity of the *A. hydrophila* enterotoxin was not neutralized by specific antisera to cholera toxin. Champsaur *et al.* (1982) and Turnbull *et al.* (1984) reported similar results for enterotoxins from clinical and environmental isolates of *A. sobria* and *A. hydrophila*. However, both Jiwa (1983) and Campbell *et al.* (1984) reported that *A. hydrophila* enterotoxin was neutralized by antisera to cholera toxin. Hostacka *et al.* (1982) reported that antisera to cholera toxin did not neutralize *A. hydrophila* enterotoxin, but antisera to *A. hydrophila* toxin partially neutralized cholera toxin. Hostacka *et al.* (1982) and Ljungh and Wadström (1983) reported that the molecular weight for *A. hydrophila* enterotoxin was 15,000 and 60,000, respectively, suggesting that they were examining different cell products. Jiwa (1983) suggested that some *A. hydrophila* isolates may be capable of producing a heat-stable enterotoxin.

It is apparent from these reports that additional work is needed to clarify the role that enterotoxin synthesis and other virulence factors play in *Aeromonas* mediated gastroenteritis. In addition to enterotoxin production, Ljungh and Wadström (1983) suggested that hemolysin, protease, and endotoxin production may influence virulence. Jiwa (1983) suggested that cell surface hydrophobicity may be a determinant of intestinal attachment. To date, the pathogenicity of *A. hydrophila* isolates has not been correlated

with the presence of a specific plasmid (Toranzo *et al.* 1983; Cumberbatch *et al.* 1979); however, additional research is needed before the potential role of plasmid-encoded virulence factors can be adequately assessed.

A limited number of researchers have attempted to correlate the toxigenicity of *Aeromonas* isolates with other biochemical markers. Boulanger *et al.* (1977) reported that cytotoxic isolates of *A. hydrophila* and *A. sobria* produced at least one hemolysin. Similarly, Cumberbatch *et al.* (1979) and Burke *et al.* (1984) observed strong correlations between cytotoxic and hemolytic activities. Cumberbatch *et al.* (1979) also reported an apparent correlation among cytotoxicity, Voges-Proskauer (VP) reaction, and lysine decarboxylase activity. Of 65 cytotoxin-positive isolates examined, 98% and 94% were lysine decarboxylase-positive and VP-positive, respectively. Among 30 cytotoxin-negative isolates, only 27% and 23% were lysine decarboxylase-positive and VP-positive, respectively. Among 12 cytotoxin-producing *A. hydrophia* strains isolated from pediatric gastroenteritis patients, Janda *et al.* (1983) found that 75% were lysine decarboxylase-positive, while only 42% and 33% of the isolates were VP-positive and hemolytic, respectively. Turnbull *et al.* (1984) reported that enterotoxigenicity correlated strongly with positive responses for VP reaction, lysine decarboxylase, gas production on glucose, gluconate oxidation, xanthine hydrolysis, and hemolysis of human erythrocytes. Burke *et al.* (1984a) observed apparent differences in salicin and arabinose fermentations and hemagglutination patterns between clinical and environmental isolates of *A. hydrophila* and *A. sobria*.

Ecology

A. hydrophila is a ubiquitous specie, being readily isolated from a variety of sources. The best known source is water (Grabow and DuPreez 1979; Hazen *et al.* 1978; Biamon and Hazen 1983; Hazen 1983). *Aeromonas* species have been reported in both untreated and chlorinated drinking water (LeChevallier *et al.* 1980, 1982; Burke *et al.* 1984b, 1984c). The organism is commonly isolated from fish (Boulanger *et al.* 1977) and shellfish (Konuma *et al.* 1975; Faghri *et al.* 1984). Incidences of human *Aeromonas* gastroenteritis have been attributed to both contaminated water (Davis *et al.* 1978; Kipperman *et al.* 1984) and fish (Kalina 1977).

While *A. hydrophila* is generally thought of as an aquatic species, it is not restricted to that environment. It is commonly isolated from the fecal material of lower animals, and has been implicated in outbreaks of bovine abortion (Wohlegemuth *et al.* 1972) and diarrhea in piglets (Dobrescu 1978). As indicated earlier, a small percentage of humans appear to be asymptomatic carriers of *A. hydrophila*. This could have significance in regard to food handlers being a potential source of the microorganism.

Only a limited number of studies have evaluated foods for the presence of aeromonads, but it appears that the genus is ubiquitously associated with the spoilage of refrigerated animal products. *A. hydrophila* has been reported to be a common contaminant of meats, poultry, and raw milk (Enfors *et al.* 1979; Grau 1981; Kielwein *et al.* 1969; Kleeberger 1975; Myers *et al.* 1982; Toule and Murphy 1978; Blickstand and Molin 1983; Eddy and Kitchell 1959; Gardner 1965). In the case of meat products, the composition of the atmosphere surrounding the product appears to influence the extent of growth by *A. hydrophila*. Enfors *et al.* (1979) reported that *A. hydrophila* was present in high numbers in meats stored in nitrogen-flushed plastic bags, but absent in carbon dioxide-flushed samples. Similarly, the organism was isolated from vacuum-packaged fresh pork (Bowen and Kominas 1979; Myers *et al.* 1982). Blickstand and Molin (1983) reported that *Aeromonas* accounted for approximately 15% of the microflora of fat surfaces of pork stored under carbon dioxide at 4°C, but was not nearly as prevalent in air-flushed packages. It appears that reducing oxygen levels surrounding refrigerated meats favors the growth of *Aeromonas,* possibly by retarding the growth of competing *Pseudomonas*. However, *Aeromonas* can grow on aerobically stored refrigerated products, particularly if the competing microflora has been reduced by cooking or some other treatment. The public health significance of *A. hydrophila* in meats has not been assessed; however, based on the limited number of surveys of *Aeromonas* isolates from fish and other sources (Boulanger *et al.* 1977; Hostacka *et al.* 1982; Jiwa 1983), it is possible that some strains are potentially enterotoxigenic.

One of the characteristics that strongly influences the potential importance of *A. hydrophila* and *A. sobria* in regards to food safety is their psychrotrophic nature. Bergey's Manual (Popoff 1984) lists *A. hydrophila* as being capable of growth over a temperature range of 0 of 41°C, and at refrigeration temperatures (4 to 7°C), the species grows at a sufficiently rapid rate as to be competitive with other psychrotrophic species associated with foods. We (Palumbo and Buchanan 1984) have recently completed a limited evaluation of the effect of temperature on the growth of five enterotoxigenic strains of *A. hydrophila* in microbiological media, and found that all tested isolates could attain reasonably high population densities within 7 to 10 days at 4°C. On the other end of the temperature range, all isolates were capable of growth at 35°C, and two of the five strains grew at 42°C. The microorganism has also been shown to grow competitively at 4 to 5°C in chicken, beef, pork, and milk (Enfors *et al.* 1979; Grau 1981; Kielwein *et al.* 1969; Toule and Murphy 1978).

Isolation and Enumeration of Foodborne Aeromonads

Techniques currently available for the detection and enumeration of *Aeromonas* largely reflect applications for stool and water samples. Various en-

teric media have been used for the isolation of the genus (Pitarangsi *et al.* 1982; Champsaur *et al.* 1982; LeChevallier *et al.* 1982); however, these techniques are not totally applicable since prime taxonomic determinants for various *Enterobacteriaceae* can be variable in *Aeromonas*. For example, Popoff and Veron (1976) concluded that lactose utilization is not a suitable characteristic for differentiating the genus.

Several direct plating media have been designed or adopted for use in detecting *A. hydrophila* from clinical or environmental samples. These detecting *A. hydrophila* from clinical or environmental samples. These include Rimler-Shotts agar (Shotts and Rimler 1973), dextrin-fuchsin-sulfite agar (Schubert 1967), DNase-toluidine blue-ampicillin agar (von Graevenitz and Zinterhofer 1970), inositol-brilliant green-bile salts agar (Schubert 1977), peptone-beef extract-glycogen agar (McCoy and Pilcher 1974), Pril-xylose-ampicillin agar (Rogol *et al.* 1979), Rippey-Cabelli agar (Rippey and Cabelli 1979), salt-starch-xylose-lysine-sodium desoxycholate agar (Roland 1977), and xylose-sodium desoxycholate-citrate agar (Millership *et al.* 1983; Shread *et al.* 1981). Trypticase soy-ampicillin broth (von Graevenitz and Zinterhofer 1970) and alkaline peptone water (Shread *et al.* 1981) have been recommended for the selective enrichment for detection of low numbers of *A. hydrophila*.

von Graevenitz and Bucher (1983) evaluated nine plating media and enrichment broths for their ability to detect *A. hydrophila* in stool specimens and correctly differentiated them from *Enterobacteriaceae* and *Pleisiomonas*. They recommended the use of alkaline peptone water as an enrichment, and either inositol-brilliant green-bile salts agar, dextrin-fuchsin-sulfite agar, xylose-sodium desoxycholate-citrate agar, or Pril-xylose-ampicillin agar as a differential plating medium. The applicability of these media for the detection and enumeration of *Aeromonas* in foods awaits future evaluation; however, preliminary studies in our laboratory (unpublished data) have suggested that a number of the media are unsuitable for quantitatively detecting aeromonads from a food matrix. Myers *et al.* (1982) reported that *A. hydrophila* could be isolated from meat samples using sorbitol-bile broth incubated at 5°C as an enrichment technique, followed by detection on pectin agar incubated at 25°C. However, this protocol needs clarification since Myers *et al.* (1982) reported that the *A. hydrophila* isolated in this manner were largely pectinolytic, while Popoff and Veron (1976) concluded that *Aeromonas* species were universally pectinase-negative.

Initial studies in our laboratory (unpublished data) have indicated that phenol red broth base-starch-ampicillin agar (Table 2) is useful for quantitatively detecting *Aeromonas* in foods. Details of this medium will be published at a later date.

Upon isolation, most investigators have employed the API 20E system

Table 2. Composition of phenol red broth-ampicillin-starch agar[1]

Phenol red broth base (Difco #0092)	16 g
Soluble starch	20 g
Agar	20 g
Ampicillin	10 mg
Distilled water	1000 ml

[1]Plates incubated for 24h at 28°C. Starch hydrolysis detected by flooding plates with ca 5 ml of Gram's iodine.

(Analytab Products, Inc., Plainview, NY) for the identification of presumptive *A. hydrophila* isolates. Waltman *et al.* (1982) reported that the API ZYM system was useful in identifying members of the *A. hydrophila* complex, and that the system would be useful in differentiating *A. hydrophila* and *A. sobria*. Kaper *et al.* (1979) developed a multi-test tube medium (AH medium) for the presumptive identification of *A. hydrophila*.

CONCLUSIONS

While there are data still needed to fully evaluate the role of *A. hydrophila* and *A. sobria* as potential causes of gastroenteritis in humans, the majority of evidence to date suggests that at least some strains of these species may turn out to be enteric pathogens. Specific information on the possibility that foods may be a significant vector for the transmission of enterotoxigenic *Aeromonas* is largely unavailable. These data are needed before an adequate assessment can be made in regard to their potential role as food poisoning organisms. Considering the psychrotrophic nature of the genus and the obvious implication of food poisoning species that can grow readily at refrigeration temperatures, it seems imperative that the information needed to assess their food safety significance be obtained at the earliest possible date. This process could be greatly accelerated if public health officials involved in the investigation of suspected food poisoning outbreaks included. *A. hydrophila* and *A. sobria* as species of interest. Until more definitive information is available concerning these microorganisms in foods, it seems prudent to recommend that individuals involved in the

manufacturing, distribution, and preparation of foods consider *A. hydrophila* and *A. sobria* as undersirable. Further, they should be cognizant that high levels of these bacteria may render their products unsafe. This would be particularly true for foods targeted for high risk segments of the population (i.e., infants, elderly, immunocompromised patients), and in this instance active steps may be needed to limit exposure to these potential enteric pathogens.

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